

10/PRB

09/869098

JCOS Rec'd PCT/PTO 21 JUN 2001

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SPECIFICATION
UCP-2 Promoter DNA

FIELD OF THE INVENTION

5 This invention relates to a novel promoter for gene expression and its use. Specifically, this invention relates to a DNA containing the promoter region of human uncoupling protein-2 (UCP-2) gene, a transformant transformed with the said DNA, and a
10 method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity.

BACKGROUND OF THE INVENTION

Uncoupling protein (UCP) is a proton transporter
15 present in the mitochondrial inner membrane. Since UCP changes intracellular energy stored as fat to heat without using other energy consuming processes, UCP is considered to play an important role in maintenance of body temperature in homeothermal animals. Because of
20 this function, UCP is considered to be an important factor that determines the efficiency of energy metabolism in homeothermal animals.

Three molecular species of uncoupling protein have been identified to date, and are called uncoupling
25 proteins-1 (UCP-1), -2 (UCP-2 or UCPH), and -3 (UCP-3).

UCP-1, the first isolated among the uncoupling protein family, is specifically expressed in brown fat cells (Line, C.S. and Klingenberg, M. (1980), FEBS Lett., 113, 299-303; Jacobsson, A. et al. (1985), J.
30 Biol. Chem., 260, 16250-16254; Bouillaud, F. et al. (1986), J. Biol. Chem., 261, 1487-1490). UCP-2 was isolated as a homologue of UCP-1, and confirmed to be widely expressed in various organs (Gimeno, R.E. et al. (1997), Diabetes, Vol. 46, 900-906; Fleury, C., et al.
35 (1997), Nature Genet., Vol. 15, 269-272). UCP-3 was

isolated as a UCP specifically expressed in muscle (Vidal-Puig, A. et al. (1997), *Biochem. Biophys. Res. Commun.*, Vol. 235, No. 1, 79-82; Boss, O. et al. (1997), *FEBS Lett.*, 408, 33-38).

- 5 Generally, UCP-1 is considered to play an important role in maintenance of body temperature in rodents and hibernants. As for the basis, the number of brown fat cells that mainly express UCP-1 is lower in large sized animals and animal species inhabiting in relatively warm weather (Rothwell, N.J. and Stock, M.J. (1979), *Nature*, Vol. 281, 31-35). Thus, in these animals including human, UCP-2 or UCP-3, not UCP-1, may mainly be responsible for the control of the normal body temperature maintenance system and energy consuming process (Hosoda, K. et al. (1998), *Obesity Research (Himan Kenkyu)*, Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), *Nature*, Vol. 387, 90-93).

- Therefore, it may be possible to adjust the energy consumption/accumulation balance by controlling the gene expression or activity of UCP-2 or UCP-3 in these animals including human (Hosoda, K. et al. (1998), *Obesity Research (Himan Kenkyu)*, Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), *Nature*, Vol. 387, 90-93). In human, enhancement of energy consumption is considered to promote consumption of not only dietary energy but also energy accumulated as fat. Accordingly, a decrease of body fat in human may lead to improvement of obesity, the major cause of lifestyle diseases which become a problem in developed countries in recent years (Fleury, C. et al. (1997), *Nature Genetics*, Vol. 15, 269-272).

- UCP-2 is also considered to be the major cause of fever observed in immunological inflammation such as infection, and inhibition of UCP-2 gene activity may reduce fever in immunological inflammation (Shigenaga,

F.R. et al. (1998), *Biochim. Biophys. Res. Commun.*, Vol. 244, No. 1, 75-78).

In animals, especially in higher animals, organs differentiate and mature upon biogenesis, and develop to exert each function. During this process, various organ-specific proteins are transiently or constantly expressed and provide the organ-specificity.

The general gene expression control system in animals includes the transcription induction system (promoter, enhancer). Promoter regions are generally located adjacent to the 5' upstream region of base sequences on chromosomes that are normally transcribed into messenger RNAs. Transcriptional regulator protein is bound to or dissociated from base sequence generally called regulator sequence in promoter regions, by which the transcription level of genes located downstream of the 3' region is regulated. Therefore, the transcriptional gene expression level can be estimated from the promoter activity to some extent. It is also known that the base sequences located downstream of the 3' region of a promoter do not affect the promoter activity in most cases. Therefore, promoter activity can be readily measured by substituting the transcribed messenger RNA for a base sequence encoding a protein with detectable enzyme activity (reporter). Recent technical innovation has made measurement of promoter activity using reporters very sensitive and simple, and measurement of promoter activity is used in drug screening and analysis of biological function.

For example, transcriptional regulatory factors of fat cell differentiation include peroxisome proliferation-activated receptor γ (PPAR γ) (Tontontz, P. et al. (1995), *Curr. Opin. Genet. Dev.*, Vol. 5, 571-576), retinoid X receptor (RXR), CCAAT/enhancer binding protein (C/EBP) (Cornelius, P., et al. (1994), *Annu.*